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ANTI-ANGIOGENIC VACCINES: SUBSTANCES AND METHODS RELATING THERETO

Field of the invention

The present invention concerns materials and methods relating to anti-angiogenic vaccines.

Background to the invention

Solid tumours are dependent on the development of an adequate blood supply for growth and spread of metastasis. This is achieved by the growth of new blood vessels through the process of angiogenesis and parasitisation of pre-existing host vessels.

Several receptor tyrosine kinases (RTK) have been identified which are associated with endothelial cell proliferation and differentiation. These comprise the vascular endothelial growth factor (VEGF) family (Class III) RTKs, Flt-1, Flt-4, and KDR; and the Tie (Tie-1) (Partanen, J., et al 1992 Mol. Cell. Biol., 12 p1698) and Tek (also designated Tie-2) (Dumont, D.J. et al., 1992 Oncogene, 8 p147) RTKs. As both Tie and Tek have unique multiple extracellular domains consisting of immunoglobulin-like loops, epidermal growth factor-like repeats, and fibronectin type III repeats they are thought to represent a new family (Class VIII) of RTKs (Plate, K.H. et al., Brain Pathology 1994 4 p207-218). The Tek gene encodes a 140 kDa polypeptide and has been cloned from embryonic murine heart, murine brain capillaries and human placenta cDNA libraries (Dumont, D.J. et al., 1992 supra., Schnurch, H. and Risau, W. 1993 Development, 119 p957; Ziegler, S.F. et al., 1993 Oncogene 8 p663).

Accumulating evidence indicates that VEGF is the central

mediator of developmental, hypoxia-mediated and tumour induced angiogenesis (Plate, K.H. et al., Brain Pathology 1994, 4 p207-218). VEGF, secreted by tumours, binds to endothelial cells which express VEGF RTKs FLT-1 and KDR. FLT-1 is expressed by non endothelial cells including monocytes (Hewett, P., Biochem. Biophys. Res Comm. 1996, 221 p697-702) making it an inappropriate target for vaccines.

Although the exact role that Tie and Tek play in angiogenesis is still unclear, Tek has recently been observed in the endothelium of murine breast tumours (Millauer, B. et al., 1996 Cancer Res. 56 p1615) and it appears that Tek plays a pivotal role in the differentiation, proliferation and survival of embryonic endothelium, as homozygous dominant-negative Tek mutants are not viable. A 1.2kb region of the murine Tek flanking sequence has been shown to act as an endothelial lineage-specific promoter during embryonic development in transgenic mice (Schlaeger, T.M. et al., 1995 Development, 121 p1089). Tek is also known to be readily upregulated on HUVEC cells exposed to tumour conditioned medium (Hewett, P.W. et al., Br. J. Cancer 1996 73 p53-53). However this is no evidence that T cells recognise receptor kinases overexpressed on endothelial cells.

VEGF-receptors and PDGF receptors of subclass III of receptor kinases show structural homology. In relation to vaccine design, immune responses need to be directed to sequences unique to the VEGF receptors.

Endothelial cells are in principal potential targets for T cell attack as they express class I MHC and can be induced to express class II MHC (Dhibjalbut S.S. et al., Journal of Immunology 1993, 151 p6248-6258). The

cytotoxicity of T cells for cerebral endothelial cells has been implicated in the breakdown of the blood brain barrier and development of inflammatory lesions in the central nervous system (Tsukada, N. et al., Autoimmunity 1994, 17 p225-232).

Summary of the invention

The present inventors have discovered that Tek has MHC binding epitopes which can bind to MHC to stimulate helper and/or cytotoxic T cell responses in vitro.

The results described herein suggest that short amino acid sequences presenting epitopes of Tek can be used as cancer vaccines to direct an immune cell response to the endothelia as evidenced by helper T cell responses including cytokine release and recruitment of non-specific effector cells such as natural killer cells or tumouricidal macrophages, stimulation of cytotoxic T cell responses or antibody responses.

Thus the present invention generally concerns agents based upon one or more of these epitopes which are in Tek for use as eg vaccines to target the endothelial cells lining the blood vessels of a tumour; methods for preparation of the agents; methods for preparation of vaccines comprising as an essential constituent such agents; vaccines comprising such agents.

Provided by the present invention are peptides and polypeptides which do not occur naturally and which consist essentially of one or more amino acid sequences that represent one or more epitopes of the Tek protein. The peptides or polypeptides, which comprise less than the full length polypeptide sequence of native Tek, can bind to MHC to stimulate a helper and/or cytotoxic T cell

immune response.

By the terms "consists essentially of", it is intended to mean that peptides or polypeptides of the present invention consist largely of one or more sequences which represent epitopes of Tek protein, with little in the way of other sequences of the native Tek protein.

By the terms "represent one or more epitopes", it is intended to mean that the sequences are identical to, or differ only in immaterial variants of the sequence of a native Tek epitope, such that it retains the function of a Tek epitope.

Thus agents for use as eg vaccines may comprise such peptides/polypeptides or DNA constructs in the form of plasmids or vectors carrying nucleic acid encoding such peptides/polypeptides. DNA constructs may have appropriate regulatory sequences to control expression of the peptide/polypeptide. Vaccine vectors are well known in the art.

A polypeptide/peptide as provided may comprise less than the full-length Tek polypeptide sequence. It may have one or more sequences of at least 5 to 7 amino acids long, often at least about 7 to 9 amino acids long, typically at least about 9 to 13 amino acids long in common with the amino acid sequence of native Tek.

Polypeptide/peptide as provided may comprise less than 50%, often less than 40 or 30%, typically less than 20%, most preferably less than 5% of the amino acid sequence of native Tek. The polypeptide/peptide may comprise 30 or less, 25 or less, typically 20 or less, preferably 15 or less, most preferably 10 or less amino acids of native

Tek protein.

Polypeptide/peptide as provided may comprise amino acid sequence presenting a single epitope of the Tek protein. Alternatively the polypeptide/peptide may comprise amino acid sequence presenting two or several epitopes of the Tek protein. The amino acid sequence may be such that the epitopes are contiguous or substantially contiguous. The amino acid sequence may be such that neighbouring epitopes are substantially devoid of the amino acid sequence that occurs between them in the native Tek protein. Thus epitopes may be coupled by employment of suitable coupling partners. Both peptidyl and non-peptidyl coupling partners are well-known in the art.

Polypeptide/peptide as provided may be a fragment of native Tek protein, or recombinantly synthesised (ie to express nucleic acid coding for the polypeptide/peptide by use of the nucleic acid in an expression system) or chemically synthesised. Polypeptides/peptides as provided may be generated wholly or partly by chemical synthesis. Thus they can be readily prepared according to well-established, standard liquid or, preferably, solid-phase peptide synthesis methods, general descriptions of which are broadly available (see, for example, in J.M. Stewart and J.D. Young, Solid Phase Peptide Synthesis, 2nd edition, Pierce Chemical Company, Rockford, Illinois (1984), in M. Bodanzsky and A. Bodanzsky, The Practice of Peptide Synthesis, Springer Verlag, New York (1984); and Applied Biosystems 430A Users Manual, ABI Inc., Foster City, California), or they may be prepared in solution, by the liquid phase method or by any combination of solid-phase, liquid phase and solution chemistry, e.g. by first completing the respective peptide portion and then, if desired and

appropriate, after removal of any protecting groups being present, by introduction of the residue X by reaction of the respective carbonic or sulfonic acid or a reactive derivative thereof.

The polypeptide/peptide may have the ability to bind to an MHC class I molecule and/or an MHC class II molecule. The polypeptide/peptide may be able to stimulate T cell proliferation. The polypeptide/peptide may have an amino acid sequence that appears within the amino acid sequence regions TEK1 (amino acids 55 to 90) TEK2 (amino acids 163 to 176) TEK3 (amino acids 345 to 362) TEK4 (amino acids 427 to 442) and TEK5 (amino acids 530 to 542) of the Tek polypeptide (see Fig.1). In particular, the polypeptide/peptide may comprise one of more of the epitope sequences Z1, Z2, Z3, Z4, Z5, Z6, Z7, Z8, Z9, Z11 and Z12 identified in Tables 1 and 4 or variant forms thereof which have substantially the same functional attributes. In particular the polypeptide/peptide may comprise the epitope sequence Z1 or Z32 identified in Tables 1 and 4 or variant forms thereof which have substantially the same functional attributes.

A variant form of an epitope sequence named above means a sequence modified by varying the sequence of amino acids eg by manipulation of encoding nucleic acid. The variation may involve insertion, addition, deletion or substitution of one or more amino acids, to provide an epitope sequence having substantially the same functional attributes of the epitope sequence from which the variant is derived.

Thus the amino acid sequence for Z1 is disclosed herein. It is also disclosed that Z1 binds HLA-A2 with a stabilisation ratio of 2.3 and is able to stimulate T-

cell proliferation. The amino acid sequence of Z1 may be slightly varied whilst retaining ability to bind HLA-A2 and stimulate T-cell proliferation.

Thus conservative variations may be made ie the substitution of one hydrophobic residue such as isoleucine, valine, leucine or methionine for another, or the substitution of one polar residue for another, such as arginine for lysine, glutamic for aspartic acid, or glutamine for asparagine.

Thus preferred such variants retain the function of the peptides from which they are derived. Such properties are mentioned above and identified herein. There may also be immunological cross-reactivity with an antibody reactive to a peptide of Tek regions TEK1 to 5 (see Fig.1) or as identified in Tables 1 and 4; sharing an epitope with a peptide of Tek regions TEK1 to 5 (see Fig.1) or as identified in Tables 1 and 4 (as determined for example by immunological cross-reactivity between the two peptides).

A variant form of a peptide from Tek regions TEK1 to 5 (see Fig.1) or as identified in Tables 1 and 4 may comprise an amino acid sequence which shares greater than about 30% sequence identity with the sequence shown, greater than about 40%, greater than about 50%, greater than about 60%, greater than about 70%, greater than about 80% or greater than about 90%. The sequence may share greater than about 60% similarity, greater than about 70% similarity, greater than about 80% similarity or greater than about 90% similarity with an amino acid sequence from Tek regions TEK1 to 5 (see Fig.1) or as identified in Tables 1 and 4. Particular amino acid sequence variants may differ from those from Tek regions

TEK1 to 5 (see Fig.1) or as identified in Tables 1 and 4 by insertion, addition, substitution or deletion of 1 amino acid, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20 amino acids.

The polypeptide/peptide may comprise two or more epitope sequences from Tek regions TEK1 to 5 (see Fig.1) or as identified in Tables 1 and 4 or two or more variants of epitope sequences from Tek regions TEK1 to 5 (see Fig.1) or as identified in Tables 1 and 4. Thus the polypeptide/peptide may comprise the Z32 epitope sequence of Table 4.

The peptide may have an amino acid sequence shown in Table 1 or Table 4.

Polypeptides/peptides as provided may be in isolated and/or purified form, free or substantially free of material with which it is naturally associated.

A polypeptide/peptide as provided may be isolated and/or purified (e.g. using an antibody) for instance after production by expression from encoding nucleic acid. They may also be generated wholly or partly by chemical synthesis. They may be used in the formulation of a composition, which may include at least one additional component, for example a pharmaceutical composition including a pharmaceutically acceptable excipient, vehicle or carrier. A composition including a polypeptide/peptide as provided may be used in prophylactic and/or therapeutic treatment as discussed below.

A polypeptide/peptide as provided may be used as an immunogen or otherwise in obtaining specific antibodies.

Antibodies are useful in eg purification and other manipulative techniques.

The production of monoclonal antibodies is well established in the art. Monoclonal antibodies can be subjected to the techniques of recombinant DNA technology to produce other antibodies or chimeric molecules which retain the specificity of the original antibody. Such techniques may involve introducing DNA encoding the immunoglobulin variable region, or the complementarity determining regions (CDRs), of an antibody to the constant regions, or constant regions plus framework regions, of a different immunoglobulin. See, for instance, EP-A-184187, GB-A-2188638 or EP-A-239400. A hybridoma producing a monoclonal antibody may be subject to genetic mutation or other changes, which may or may not alter the binding specificity of antibodies produced.

The provision of the novel polypeptides/peptides enables for the first time the production of antibodies able to bind specifically to them. Accordingly, a further aspect of the present invention provides an antibody able to bind specifically to a peptide Tek regions TEK1 to 5 (see Fig.1) or as identified in Tables 1 and 4 or variant peptide thereof as discussed. Such an antibody may be specific in the sense of being able to distinguish between the peptide it is able to bind and other peptides for which it has no or substantially no binding affinity (e.g. a binding affinity of about 1000x worse). Specific antibodies bind an epitope on the molecule which is either not present or is not accessible on other molecules. Antibodies according to the present invention may be specific for the wild-type polypeptide. Antibodies are also useful in purifying the peptides or polypeptides to which they bind, e.g. following

production by recombinant expression from encoding nucleic acid.

Preferred antibodies according to the invention are isolated, in the sense of being free from contaminants such as antibodies able to bind other polypeptides and/or free of serum components. Monoclonal antibodies are preferred for some purposes, though polyclonal antibodies are within the scope of the present invention.

Antibodies may be obtained using techniques which are standard in the art. Methods of producing antibodies include immunising a mammal (e.g. mouse, rat, rabbit, horse, goat, sheep or monkey) with the protein or a fragment thereof. Antibodies may be obtained from immunised animals using any of a variety of techniques known in the art, and screened, preferably using binding of antibody to antigen of interest. For instance, Western blotting techniques or immunoprecipitation may be used (Armitage et al, Nature, 357:80-82, 1992). Isolation of antibodies and/or antibody-producing cells from an animal may be accompanied by a step of sacrificing the animal.

As an alternative or supplement to immunising a mammal with a peptide, an antibody specific for a protein may be obtained from a recombinantly produced library of expressed immunoglobulin variable domains, e.g. using lambda bacteriophage or filamentous bacteriophage which display functional immunoglobulin binding domains on their surfaces; for instance see WO92/01047. The library may be naive, that is constructed from sequences obtained from an organism which has not been immunised with any of the proteins (or fragments), or may be one constructed using sequences obtained from an organism which has been

exposed to the antigen of interest.

Antibodies according to the present invention may be modified in a number of ways. Indeed the term "antibody" should be construed as covering any binding substance having a binding domain with the required specificity. Thus the invention covers antibody fragments, derivatives, functional equivalents and homologues of antibodies, including synthetic molecules and molecules whose shape mimics that of an antibody enabling it to bind an antigen or epitope.

Example antibody fragments, capable of binding an antigen or other binding partner are the Fab fragment consisting of the VL, VH, Cl and CH1 domains; the Fd fragment consisting of the VH and CH1 domains; the Fv fragment consisting of the VL and VH domains of a single arm of an antibody; the dAb fragment which consists of a VH domain; isolated CDR regions and F(ab')2 fragments, a bivalent fragment including two Fab fragments linked by a disulphide bridge at the hinge region. Single chain Fv fragments are also included.

Antibodies may be humanised if appropriate ie CDRs from a non-human source grafted onto human framework regions, typically with the alteration of some of the framework amino acid residues.

A hybridoma producing a monoclonal antibody for the peptides herein may be subject to genetic mutation or other changes. It will further be understood by those skilled in the art that a monoclonal antibody can be subjected to the techniques of recombinant DNA technology to produce other antibodies or chimeric molecules which retain the specificity of the original antibody. Such

techniques may involve introducing DNA encoding the immunoglobulin variable region, or the complementarity determining regions (CDRs), of an antibody to the constant regions, or constant regions plus framework regions, of a different immunoglobulin. See, for instance, EP-A-184187, GB-A-2188638 or EP-A-0239400. Cloning and expression of chimeric antibodies are described in EP-A-0120694 and EP-A-0125023.

Hybridomas capable of producing antibody with desired binding characteristics are within the scope of the present invention, as are host cells, eukaryotic or prokaryotic, containing nucleic acid encoding antibodies (including antibody fragments) and capable of their expression. The invention also provides methods of production of the antibodies including growing a cell capable of producing the antibody under conditions in which the antibody is produced, and preferably secreted.

The reactivities of antibodies on a sample may be determined by any appropriate means. Tagging with individual reporter molecules is one possibility. The reporter molecules may directly or indirectly generate detectable, and preferably measurable, signals. The linkage of reporter molecules may be directly or indirectly, covalently, e.g. via a peptide bond or non-covalently. Linkage via a peptide bond may be as a result of recombinant expression of a gene fusion encoding antibody and reporter molecule.

One favoured mode is by covalent linkage of each antibody with an individual fluorochrome, phosphor or laser dye with spectrally isolated absorption or emission characteristics. Suitable fluorochromes include fluorescein, rhodamine, phycoerythrin and Texas Red.

Suitable chromogenic dyes include diaminobenzidine.

Other reporters include macromolecular colloidal particles or particulate material such as latex beads that are coloured, magnetic or paramagnetic, and biologically or chemically active agents that can directly or indirectly cause detectable signals to be visually observed, electronically detected or otherwise recorded. These molecules may be enzymes which catalyse reactions that develop or change colours or cause changes in electrical properties, for example. They may be molecularly excitable, such that electronic transitions between energy states result in characteristic spectral absorptions or emissions. They may include chemical entities used in conjunction with biosensors. Biotin/avidin or biotin/streptavidin and alkaline phosphatase detection systems may be employed.

The mode of determining binding is not a feature of the present invention and those skilled in the art are able to choose a suitable mode according to their preference and general knowledge.

An antibody may be provided in a kit, which may include instructions for use of the antibody, e.g. in determining the presence of a particular substance in a test sample. One or more other reagents may be included, such as labelling molecules, buffer solutions, elutants and so on. Reagents may be provided within containers which protect them from the external environment, such as a sealed vial.

Figure 1 discloses areas (TEK1 to 5) of the Tek polypeptide potentially containing T cell epitopes and Tables 1 and 4 provide amino acids for useful peptides

from the areas TEK1 to 5 the inventors have discovered as being of interest as effective agents of anti-cancer vaccines. Thus the present invention provides nucleic acid molecules which encode polypeptides/peptides as provided above. Nucleotide sequences for peptides of Tek regions TEK1 to 5 (see Fig.1) or as identified in Tables 1 and 4 or variants thereof may be readily produced applying common knowledge of the genetic code. Nucleic acid encoding a peptide which is an amino acid sequence variant of a peptide sequence of Tek regions TEK1 to 5 (see Fig.1) or as identified in Tables 1 and 4 are provided.

Generally nucleic acid according to the present invention is provided as an isolate, in isolated and/or purified form, or free or substantially free of material with which it is naturally associated, such as free or substantially free of nucleic acid coding for sequences flanking sequences of interest, except possibly one or more regulatory sequence(s) for expression. Nucleic acid may be wholly or partially synthetic and may include genomic DNA, cDNA or RNA. Where nucleic acid according to the invention includes RNA, reference to the sequence shown should be construed as reference to the RNA equivalent, with U substituted for T.

Nucleic acid sequences as provided and/or accompanying regulatory elements can be readily prepared by the skilled person using the information and references contained herein and techniques known in the art (for example, see Sambrook, Fritsch and Maniatis, "Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory Press, 1989, and Ausubel et al, Short Protocols in Molecular Biology, John Wiley and Sons, 1992). These techniques include (i) the use of the

polymerase chain reaction (PCR) to amplify samples of such nucleic acid, e.g. from genomic sources, (ii) chemical synthesis, or (iii) preparing cDNA sequences. Modifications to the sequences can be made, e.g. using site directed mutagenesis, to lead to the expression of modified peptide/polypeptide or to take account of codon preference in the host cells used to express the nucleic acid.

In order to obtain expression of nucleic acid sequences as provided, the sequences can be incorporated in a vector having control sequences operably linked to control its expression. The vectors may include other sequences such as promoters or enhancers to drive the expression of the inserted nucleic acid, nucleic acid sequences so that polypeptide is produced as a fusion and/or nucleic acid encoding secretion signals so that the polypeptide/peptide produced in the host cell is secreted from the cell. Polypeptide/peptide can then be obtained by transforming the vectors into host cells in which the vector is functional, culturing the host cells so that the polypeptide/peptide is produced and recovering (eg by use of an antibody) the polypeptide/peptide from the host cells or the surrounding medium. Prokaryotic and eukaryotic cells are used for this purpose in the art, including strains of E. coli, yeast, and eukaryotic cells such as COS or CHO cells. The choice of host cell can be used to control the properties of the polypeptide/peptide expressed in those cells, e.g. controlling where the polypeptide/peptide is deposited in the host cells or affecting properties such as its glycosylation.

PCR techniques for the amplification of nucleic acid are described in US Patent No. 4,683,195. In general, such

techniques require that sequence information from the ends of the target sequence is known to allow suitable forward and reverse oligonucleotide primers to be designed to be identical or similar to the polynucleotide sequence that is the target for the amplification. PCR comprises steps of denaturation of template nucleic acid (if double-stranded), annealing of primer to target, and polymerisation. The nucleic acid probed or used as template in the amplification reaction may be genomic DNA, cDNA or RNA. PCR can be used to amplify specific sequences from genomic DNA, specific RNA sequences and cDNA transcribed from mRNA, bacteriophage or plasmid sequences. The Tek nucleic acid sequences (see Figs.1 and 2 and Tables 1 and 4) herein readily allow the skilled person to design PCR primers to identify, isolate or prepare polypeptides/peptides of the invention. References for the general use of PCR techniques include Mullis et al, Cold Spring Harbor Symp. Quant. Biol., 51:263, (1987), Ehrlich (ed), PCR technology, Stockton Press, NY, 1989, Ehrlich et al, Science, 252:1643-1650, (1991), "PCR protocols; A Guide to Methods and Applications", Eds. Innis et al, Academic Press, New York, (1990).

The nucleic acid sequences coding for polypeptides/peptides as provided are useful for identifying nucleic acid of interest (and which may be according to the present invention) in a test sample. The present invention provides a method of obtaining nucleic acid of interest, the method including hybridisation of a probe having sequence coding for a peptide of Tek regions TEK1 to 5 (see Fig.1) or as identified in Tables 1 and 4 or a complementary sequence, to target nucleic acid.

Hybridisation is generally followed by identification of successful hybridisation and isolation of nucleic acid which has hybridised to the probe, which may involve one or more steps of PCR.

Nucleic acid according to the present invention is obtainable using one or more oligonucleotide probes or primers designed to hybridise with nucleic acid sequence coding for a peptide of Tek regions TEK1 to 5 (see Fig.1) or as identified in Tables 1 and 4. A primer may be used in conjunction with one or more oligonucleotides designed to hybridise to a sequence in a cloning vector within which target nucleic acid has been cloned, or in so-called "RACE" (rapid amplification of cDNA ends) in which cDNA's in a library are ligated to an oligonucleotide linker and PCR is performed using a primer which hybridises with the template sequence of interest and a primer which hybridises to the oligonucleotide linker.

On the basis of amino acid sequence information, oligonucleotide probes or primers may be designed, taking into account the degeneracy of the genetic code, and, where appropriate, codon usage of the organism from the candidate nucleic acid is derived. An oligonucleotide for use in nucleic acid amplification may have about 10 or fewer codons (e.g. 6, 7 or 8), i.e. be about 30 or fewer nucleotides in length (e.g. 18, 21 or 24). Generally specific primers are upwards of 14 nucleotides in length, but not more than 18-20. Those skilled in the art are well versed in the design of primers for use processes such as PCR.

Nucleic acid according to the present invention may be provided as part of a kit, e.g. in a suitable container such as a vial in which the contents are protected from

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the external environment.

A convenient way of producing a polypeptide/peptide according to the present invention is to express nucleic acid encoding it in an expression system. The use of expression systems has reached an advanced degree of sophistication today.

Accordingly, the present invention also encompasses a method of making a polypeptide/peptide (as disclosed), the method including expression from nucleic acid encoding the polypeptide/peptide (generally nucleic acid according to the invention). This may conveniently be achieved by growing in culture a host cell containing a vector carrying the nucleic acid, under appropriate conditions which cause or allow expression of the polypeptide/peptide. Expression may be in in vitro systems, such as reticulocyte lysate.

Systems for cloning and expression of a polypeptide/peptide in a variety of different host cells are well known. Suitable host cells include bacteria, eukaryotic cells such as mammalian and yeast, and baculovirus systems. Mammalian cell lines available in the art for expression of a heterologous polypeptide include Chinese hamster ovary cells, HeLa cells, baby hamster kidney cells, COS cells and many others. A common, preferred bacterial host is E. coli.

Thus a further aspect of the present invention provides a vector comprising nucleic acid as provided.

Suitable vectors can be chosen or constructed, containing appropriate regulatory sequences, including promoter sequences, terminator fragments, polyadenylation

sequences, enhancer sequences, marker genes and other sequences as appropriate. Vectors may be plasmids, viral e.g. 'phage, or phagemid, as appropriate. Suitable virus vectors include herpesviruses, adenoviruses, poxviruses and retroviruses, as well as other such viruses commonly used in the art. For further details see, for example, Molecular Cloning: a Laboratory Manual: 2nd edition, Sambrook et al., 1989, Cold Spring Harbor Laboratory Press. Many known techniques and protocols for manipulation of nucleic acid, for example in preparation of nucleic acid constructs, mutagenesis, sequencing, introduction of DNA into cells and gene expression, and analysis of proteins, are described in detail in Current Protocols in Molecular Biology, Ausubel et al. eds., John Wiley & Sons, 1992.

A plasmid comprising nucleic acid as provided may be used as an immunogen (ie an anti-cancer vaccine) and injected i.m or i.d to stimulate direction a T cell response to the endothelial cells of a tumour.

A further aspect of the present invention provides a host cell containing nucleic acid as provided. The nucleic acid may be integrated into the genome (e.g. chromosome) of the host cell. Integration may be promoted by inclusion of sequences which promote recombination with the genome, in accordance with standard techniques. The nucleic acid may be on an extra-chromosomal vector within the cell.

A still further aspect provides a method which includes introducing nucleic acid as provided into a host cell. The introduction, which may (particularly for *in vitro* introduction) be generally referred to without limitation as "transformation", may employ any available technique.

For eukaryotic cells, suitable techniques may include calcium phosphate transfection, DEAE-Dextran, electroporation, liposome-mediated transfection and transduction using retrovirus or other virus, e.g. vaccinia or, for insect cells, baculovirus. For bacterial cells, suitable techniques may include calcium chloride transformation, electroporation and transfection using bacteriophage. As an alternative, direct injection of the nucleic acid could be employed.

Marker genes such as antibiotic resistance or sensitivity genes may be used in identifying clones containing nucleic acid of interest, as is well known in the art.

The introduction may be followed by causing or allowing expression from the nucleic acid, e.g. by culturing host cells (which may include cells actually transformed although more likely the cells will be descendants of the transformed cells) under conditions for expression of the gene, so that the encoded polypeptide/peptide is produced. If the polypeptide/peptide is expressed coupled to an appropriate signal leader peptide it may be secreted from the cell into the culture medium. Following production by expression, the expression product may be isolated and/or purified from the host cell and/or culture medium, as the case may be, and subsequently used as desired, e.g. in the formulation of a composition which may include one or more additional components, such as a pharmaceutical composition which includes one or more pharmaceutically acceptable excipients, vehicles or carriers (e.g. see below).

A host cell containing nucleic acid according to the present invention, e.g. as a result of introduction of nucleic acid into the cell or into an ancestor of the

cell and/or genetic alteration of the sequence endogenous to the cell or ancestor (which introduction or alteration may take place in vivo or ex vivo), may be comprised (e.g. in the soma) within an organism which is an animal, particularly a mammal, which may be human or non-human, such as rabbit, guinea pig, rat, mouse or other rodent, cat, dog, pig, sheep, goat, cattle or horse, or which is a bird, such as a chicken. Genetically modified or transgenic animals or birds comprising such a cell are also provided as further aspects of the present invention.

Thus host cells may be used as a nucleic acid factory to replicate the nucleic acid of interest in order to generate large amounts of it. Multiple copies of nucleic acid of interest may be made within a cell when coupled to an amplifiable gene such as DHFR. Host cells transformed with nucleic acid of interest, or which are descended from host cells into which nucleic acid was introduced, may be cultured under suitable conditions, e.g. in a fermenter, taken from the culture and subjected to processing to purify the nucleic acid.

The skilled person can use the techniques described herein and others well known in the art to produce large amounts of the polypeptides/peptides of the invention for use as pharmaceuticals, in the developments of drugs and for further study into their properties and role in vivo.

As mentioned above the polypeptides/peptides provided and also nucleic acid constructs (eg viral vaccine) can be formulated in pharmaceutical compositions. These compositions may in addition comprise a pharmaceutically acceptable excipient, carrier, buffer, stabiliser or other materials well known to those skilled in the art.

Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material may depend on the route of administration, e.g. oral, intravenous, cutaneous or subcutaneous, nasal, intramuscular, intraperitoneal routes. The formulation may be liquid and ordinarily a physiologic salt solution containing non-phosphate buffer at pH 6.8-7.6, or may be lyophilized powder.

Pharmaceutical compositions for oral administration may be in tablet, capsule, powder or liquid form. A tablet may include a solid carrier such as gelatin or an adjuvant. Liquid pharmaceutical compositions generally include a liquid carrier such as water, petroleum, animal or vegetable oils, mineral oil or synthetic oil. Physiological saline solution, dextrose or other saccharide solution or glycols such as ethylene glycol, propylene glycol or polyethylene glycol may be included.

For intravenous, cutaneous or subcutaneous injection, or injection at the site of affliction, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride Injection, Ringer's Injection, Lactated Ringer's Injection. Preservatives, stabilisers, buffers, antioxidants and/or other additives may be included, as required.

Administration is preferably in a "prophylactically effective amount" or a "therapeutically effective amount" (as the case may be, although prophylaxis may be

considered therapy), this being sufficient to show benefit to the individual. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of what is being treated. Prescription of treatment, e.g. decisions on dosage etc, is within the responsibility of general practitioners and other medical doctors, and typically takes account of the disorder to be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners. Examples of the techniques and protocols mentioned above can be found in Remington's Pharmaceutical Sciences, 16th edition, Osol, A. (ed), 1980.

A polypeptide/peptide as taught may be prepared for administration by mixing at the desired degree of purity with adjuvants or physiologically acceptable carriers, i.e. carriers which are non toxic to recipients at the dosages and concentrations employed. Adjuvants and carriers are substances that in themselves share no immune epitopes with the target antigen, but which stimulate the immune response to the target antigen. Ordinarily, this will entail combining active ingredient with buffers, low molecular weight (less than about 10 residues) polypeptides, proteins, amino acids, carbohydrates including glucose or dextrans, cheating agents such as EDTA, and other excipients. Freunds adjuvant (a mineral oil emulsion) commonly has been used for this purpose, as have a variety of toxic microbial substances such as mycrobacterial extracts and cytokines such as tumour necrosis factor and interferon gamma. Other adjuvants for vaccination are disclosed in EP-A-0745388, WO97/01330 and EP-A-0781559. Carriers can also act as adjuvants, but are generally distinguished from

adjuvants in that carriers comprise water insoluble macromolecular particulate structures which aggregate the antigen, typical carriers include aluminum hydroxide, latex particles, bentonite and liposomes.

A composition may be administered alone or in combination with other treatments, either simultaneously or sequentially dependent upon the condition to be treated.

Administration may be via injection (intramuscular or subcutaneous) intravenous delivery, or delivery through catheter or other surgical tubing. Alternative routes include tablets and the like, commercially available nebulizers for liquid formulations, and inhalation of lyophilized or aerosolized receptors. Liquid formulations may be utilized after reconstitution from power formulations.

The polypeptides/peptides taught may also be administered via microspheres, liposomes, other microparticulate delivery systems or sustained release formulations placed in certain tissues including blood. Suitable examples of sustained release carriers include semipermeable polymer matrices in the form of shaped articles, e.g. suppositories, or microcapsules. Implantable or microcapsular sustained release matrices include polylactides (US Patent No:3,773,919, EP-A-0058481) copolymers of L-glutamic acid and gamma ethyl-L-glutamate (Sidman et al, Biopolymers 22(1): 547-556, 1985), poly(2-hydroxyethyl-methacrylate) or ethylene vinyl acetate (Langer et al, J. Biomed. Mater. Res. 15:167-277, 1981, and Langer, Chem. Tech. 12:98-105, 1982). Liposomes containing the polypeptides are prepared by well-known methods: DE 3,218,121A; Epstein et al, PNAS USA, 82:3688-3692, 1985; Hwang et al, PNAS USA, 77:4030-4034,

1980; EP-A-0052522; E-A-0036676; EP-A-0088046; EP-A-0143949; EP-A-0142541; JP-A-83-11808; US Patent Nos 4,485,045 and 4,544,545. Ordinarily, the liposomes are of the small (about 200-800 Angstroms) unilamelar type in which the lipid content is greater than about 30 mol. % cholesterol, the selected proportion being adjusted for the optimal rate of the polypeptide leakage.

The vaccination dose will be dependent upon the properties of the vaccine employed, e.g. its binding activity and in vivo plasma half-life, the concentration of the polypeptide in the formulation, the administration route, the site and rate of dosage, the clinical tolerance of the patient involved, the pathological condition afflicting the patient and the like, as is well within the skill of the physician. For example, doses of 300 μg of polypeptide per patient per administration are preferred, although dosages may range from about 10 μg-1 mg per dose. Different dosages are utilized during a series of sequential inoculations; the practitioner may administer an initial inoculation and then boost with relatively smaller doses of vaccine.

The vaccine compositions of the invention can be administered in a variety of ways and to different classes of recipients.

At least three separate inoculations with the polypeptides/peptides may be administered, with a second inoculation being administered more than two, preferably three to eight, and more preferably approximately four weeks following the first inoculation. A third inoculation may be administered several months later than the second "boost" inoculation, preferably at least more than five months following the first inoculation, more

preferably six months to two years following the first inoculation, and even more preferably eight months to one year following the first inoculation. Periodic inoculations beyond the third are also desirable to enhance the patient's "immune memory". See Anderson et al, J Infectious Diseases 160 (6):960-969, Dec.1989 and the references therein. Generally, infrequent immunizations with polypeptides spaced at relatively long intervals is more preferred than frequent immunizations in eliciting maximum antibody responses, and in eliciting a protective effect.

Alternatively, targeting therapies may be used to deliver the active agent more specifically to certain types of cell, by the use of targeting systems such as antibody or cell specific ligands. Targeting may be desirable for a variety of reasons; for example if the agent is unacceptably toxic, or if it would otherwise require too high a dosage, or if it would not otherwise be able to enter the target cells.

Instead of administering polypeptides/peptides directly, their production in target cells can be achieved by expression from an encoding gene introduced into the cells, eg in a viral vector (a variant of the VDEPT technique - see below). The vector could be targeted to the specific cells to be treated, or it could contain regulatory elements which are switched on more or less selectively by the target cells. Vectors such as viral vaccine vectors have been used in the prior art. In particular, a number of viruses have been used poxviruses such as vaccinia virus. Alternatives are well known. A variety of vectors, both viral vectors and plasmid vectors are known in the art, see US Patent No. 5,252,479 and WO93/07282.

The agent may be administered in a precursor form, for conversion to the active form by an activating agent produced in, or targeted to, the cells to be treated. This type of approach is sometimes known as ADEPT or VDEPT, the former involving targeting the activating agent to the cells by conjugation to a cell-specific antibody, while the latter involves producing the activating agent, e.g. an enzyme, in a vector by expression from encoding DNA in a viral vector (see for example, EP-A-415731 and WO 90/07936).

Further vaccination may be achieved by simple injection of naked DNA in accordance with recent reports which show that injection of naked DNA encoding an immunogen either directly into muscle cells or as DNA coated microgold particles via a gene gun into skin cells. In the latter case some beads are taken up by dendritic cells and transported to the draining lymph nodes. This procedure has resulted in prolonged humoral and cell mediated immunity. It therefore offers all the advantages of live viral vaccines as protein is continuously produced within antigen presenting cells, but is safer as the DNA is engineered to contain only the genes for the immunogenic protein. DNA encoding the protein/polypeptide of interest is cloned into a suitable expression vector such as the eukaryotic expression vector pCR3 (Invitrogen). Inclusion of signal sequences will be used to target the translated protein to class I or class II compartments. In relation to the treatment of tumours, vaccines which direct an immune response to endothelia offer a number of advantages over vaccines targeting a response to epithelia.

Firstly since each capillary provides oxygen and nutrients for thousands of tumour cells, even limited

damage to the tumour vasculature would result in large scale destruction of tumour cells. Secondly endothelial cells are directly accessible to the immune system and thirdly the problems of antigen heterogencity, MHC loss and resistance to apoptosis which are associated with mutant epithelial cells are unlikely to occur where the immune response is directed to normal endothelial cells. Finally, tumour endothelial cells overexpress molecules including a range of tyrosine kinase receptors such as Tek.

In order that the present invention is better understood there follows a description of the experimental work underlying the discovery. The description also provides detailed information relating to the identification, provision and use of peptide epitopes which the inventors propose are valuable in the preparation of useful anti-tumour vaccines.

Reference is made to the accompanying figures which are described below.

Brief description of the drawings

Figure 1. Shows the sequence for Tek and identifies five potential T cell epitopes in the immunoglobulin-like loops and around the EGF-like and fibronectin type III-like domains.

Figure 2. Shows Genbank sequence alignments which indicate restriction of the potential T cell epitopes to Tek.

Figure 3. Shows the results of an experiment to measure the ability of the peptides identified to bind to HLA-A2 (measured by quantifying the surface expression of HLA-A2

molecules on T2 cell line by indirect immunofluorescence with W6/32 monoclonal antibody and flow cytometric analysis; peptides were B6 hepatitis surface antigen peptide (FLPSDFFPSV), D1 MAGE HLA-A1 peptide (EVDPIGHLY) and Tek peptides Z1 and Z12).

Figure 4. Shows the results of an experiment to measure T cell proliferation responses of volunteer 1.

Figure 5. Hydropathicity Profile for Tek.

Detailed description of the drawings and exemplification of the invention

EXPERIMENTAL

METHODS

Potential T cell epitopes have been identified within overexpressed tyrosine kinases by motif analysis and/or overlapping peptide analysis. These epitopes have been confirmed *in vitro* by binding to human MHC and stimulation of helper and cytotoxic T cell responses.

Motif Analysis

Different sets of peptides are displayed by individual class I isoforms with each MHC class I molecule having its individual peptide specificity, or motif, usually characterised by a defined number of amino acids and two anchor residues. The latter represent interaction sites with the MHC binding groove, as revealed by crystallography. One anchor is at the C-terminus of peptides and is either hydrophobic or positively charged: a second anchor is at position 2, 3, or 5 (Germain R.N. The Immunologist 1995, 3 p185-190).

MHC class II molecules also display allele-specific peptide ligand motifs. Their determination has been more difficult than for class 1 because class II ligands have their N- and C- termini protruding out of the MHC groove, which tightly holds a nonamer stretch in the middle of 12 to 25-mer peptides. Analysis of over 9,000 peptides eluted from MHC molecules has identified over 200 motifs binding to a wide range of MHC phenotypes (Brusic, V. et al., Nucl. Acids Res. 1994, 22 p3663-3665). These motifs can be used to analyse proteins to identify potential T cell epitopes. Although having the correct anchor residue (motif) is necessary for MHC binding it is by no means sufficient, as non-anchor residues can exert important effects on both MHC peptide binding and T cell stimulation.

The present inventors have analysed the structure of Tek in order to identify areas that appear unique to the receptors and which display a potential for T-cell epitope generation. The inventors have identified five potential epitopes in the immunoglobulin-like loops of Tek and around the EGF-like and fibronectin type III-like domains (Fig.1). These potential epitopes have been rechecked against the Genbank database. It appears that the epitopes are restricted to Tek (Fig.2). Peptides based on these results have been synthesised and tested for MHC binding.

Table 1 identifies potential T cell epitopes within Tek.

Table 1

Amino Acid Sequences of the Peptides Identified as Potential T Cell Epitopes Within Tek. The Tek region is as specified in Figure 1.

Peptide	Amino acid sequence	TEK region
Z9	GMVEKPFNI	TEK4
Z5	RMTPKIVDL	TEK3
Z1	LMNQHQDPL	TEK1
Z3	TIGRDFEAL	TEK1
Z2	NQHQDPLEV	TEK1
Z4	PRHEVPDIL	TEK2
Z6	KIVDLPDHI	TEK3
Z8	GIPRMTPKIV	TEK3
Z11	NLHPREQYV	TEK aa 606-614
Z12	ILINSLPLV	TEK aa 27-36
Z7	IVDLPDHIEV	TEK3 aa 353-362

Peptides of the table were synthesised and tested for binding to HLA-A2 molecules on the T2 cell line.

Binding of Peptides to HLA-A2

The ability of peptides to bind to HLA-A2 was measured by quantifying the surface expression of HLA-A2 molecules on T2 cell line. These cells originate from a TAP deficient cell line which can not process peptides. MHC class I molecules are unstable in the absence of peptide and therefore this cell line only expresses 20-40% of the MHC it produces. Incubation of T2 cells with MHC binding peptides can stabilise MHC and enhance surface expression (Celis, E. et al., Proc. Natl. Acad. Sci. USA 1994, 91

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p2105-9).

T2 cells were harvested and washed once in serum free (S/F) RPMI. Cells were aliquoted into 96 U well plates at 2 x 10^5/well in 100μl of S/F RPMI. Peptides (0.1-1000μg/ml) were added and left at 26°C in a 5% CO_2 incubator overnight. Expression of HLA molecules was measured by indirect immunofluoresence and results were analysed by flow cytometry. Unbound peptide was removed by washing in S/F RPMI. W6/32 (anti-HLA,A,B,C) antibody was added at 1/100 dilution in 100μl of S/F RPMI for 45 minutes on ice. Cells were washed once in S/F RPMI and then incubated on ice for 45 minutes with 100μl of rabbit anti mouse FITC added (1/1000 dilution in S/F RPMI). Cells were washed and fixed in 800μl of cellfix (1 in 10 dilution with water).

The results are summarised in Table 2 below.

Table 2

Stabilisation of HLA-A2 on the Surface of T2 Cells by Incubation with Peptides.

Peptide	HLA-A2 stabilisation ratio[a]
Z1	2.3
Z2	2.2
Z6	1.9
Z12	1.8
Z5	1.8
Z11	1.6
Z9	1.5
Z3	1.5
Z8	1.3
Z4	1.0

a) stabilisation ratio, Fluorescence in the presence of peptide/fluorescence without peptide.

Of the ten peptides only one (Z4) failed to bind to HLA-A2. Five of the peptides (Z1, Z2, Z6, Z12, Z5) showed strong binding as indicated by a stabilisation ratio >1.7. Three of the peptides (Z11, Z9, Z3) showed moderate binding as indicated by a stabilisation ratio of 1.5 to 1.7. One peptide (Z8) showed weak binding (stabilisation ratio of 1.3).

The two peptides (Z1 and Z12) which gave high stabilisation ratios were incubated with T2 cells at varying concentrations. The results for this experiment are shown in Fig.3. Z1 showed the highest affinity with 50% of maximum HLA stabilisation at 5μg/ml whereas

20μg/ml of Z12 were required.

T Cell Responses

Three of these TEK peptides (Z1, Z3, Z7) were investigated for stimulation of T cell proliferation. Blood samples were obtained from healthy volunteers. Peripheral blood mononuclear cells (PBMC) were obtained following centrifugation on Ficoll-Paque (Pharmacia Biotechnology Ltd., Milton Keynes, UK) at 220g for 30 minutes. Cells were cultured in RPMI (Sigma) with 5% human pre-screened male AB^+ and 5mM HEPES (Sigma) as follows: $1x10^6$ mononuclear cells/ml or $1x10^6$ separated T cells/ml with $1x10^5$ adherent cells/ml, in 2ml volumes in a 24 well plate (Nunc (Gibco) Life Technologies, Paisley, UK) with the appropriate concentration of antigen. The following antigens were used, at previously determined optimal concentrations: PPD (Statens Seruminstitut, Copenhagen, Denmark) at 10mg/ml; KLH (Calbiochem, Nottingham, UK) at 50mg/ml and the Tek peptides at 20mg/ml. Two ml cultures were sampled at different time points, between days 4 to 10, by transferring three 100ml aliquots from each well into 96 well microtitre plates and pulsing with 0.5mCi ^3H-thymidine/well for 6 hours. Samples were harvested onto printed filter mats (Wallac, Milton Keynes, UK) by means of a cell harvester (Skatron, Oslo, Norway), and ^3H-thymidine incorporation was measured using a b plate counter.

Enrichment of CD45RA+ cells: antigen presenting cells (APC) were obtained by adherence of PBMC in $175cm^3$ flasks for one hour at 37°C. CD45RA+ enriched T cells were negatively selected from the non-adherent cells using immunomagnetic depletion with sheep-anti-mouse IgG-coated Dynabeads (Dynal) previously incubated with an anti-CD8

antibody (Dynal).

The peptides Z1, Z3, Z7 stimulated blastogenesis responses *in vitro* on naive lymphocytes of the appropriate haplotype. The results are presented in Table 3 below.

Table 3

Proliferation Responses of Human T Cells to Tek peptides Z1, Z3, Z7 and Z32.

Volunteer	Day of peak response	Control	Z32 (cpm)	Z1	Z3	Z7	HLA-DR type
1	9	447	nd	3197	566	2314	1,4
2	8	1957	8538				3;
3	7	12920	37685				3,7
4	6	644	7785				2:11
5	6	1549	2638				3,5
6	7	2548	4180				1,13

A 20 amino acid peptide (Z32) from Tek region 1 which encompasses both the Z1 and Z3 sequences was also synthesised and screened for blastogenesis responses. The sequence for this peptide is shown in Table 4 below.

The inventors suspected that this peptide would stimulate proliferation in donors of HLA-DR1, 3, 4, 7, 8 haplotypes and possibly in most donors as it has a promiscuous pan DR binding motif (Chicz, R.M. et al., J. Exp. Med. 1993, 178 p27-47). The results are shown in Table 4 below.

Table 4

The Z32 Peptide and the Sequences Thereof which are Predicted to Bind to the Specified MHC Haplotypes.

I	T	I	G	R	D	F	E	A	L	M	N	Q	H	Q	D	P	L	E	V
		DR3							DR3										
									A2,A24,PanDR,DR1(Z1)										
	A2,H-2kb(Z3)																		
A2,A3,PanDR,DR1										A2(Z2)									
						DR1													
									DR8										
				A3,A11															
			A3																
	B8																		
		B27																	
						DR4													
						DR7													

All of the donors tested responded to the Z32 peptide. Typical proliferation responses from donor 1 to Tek peptides and the recall antigen PPD or the primary antigen KLH are shown in Fig.4.

The binding of Z32 peptide to HLA-A2, 3, 11, 24, B8 and B27 may be investigated by CTL assays. CTL activity is screened against a range of chromium labelled HLA-A matched target cells. Initially CTLs are screened against peptide pulsed lymphocytes to verify the induction of CTLs. CTL activity is then verified against cells transfected with mini gene or truncated gene constructs to demonstrate target antigen processing and presentation by MHC class I antigens. CTL activity is

screened against HUVEC induced to express high levels of Tek by tumour conditioned medium to verify that recognition by peptide induced CTLS of endogenously processed target antigens.

Immunisation with Tek specific peptides will induce antibodies which bind to tumour endothelial cells and promote coagulation and thrombosis. The sequence of Tek was analysed for hydropathicity (Fig. 5). Antibodies are more likely to bind to hydrophilic areas of antigens, in particular to regions which change from hydrophobic to hydrophilic. The Z32 peptide spans such a region.

Z32 peptide (or other peptides) can be used to immunise a suitable animal in accordance with standard procedures. Serum is screened for an antibody reaction with the Tek protein.

The identification of stimulating epitopes from angiogenic targets allows the design of vaccines for the effective generation of cytotoxic and helper T cells. The immunogen may be in the form of antigen, anti-idiotypic antibody or specific epitopes. The immunogen may be presented as either protein/polypeptide/peptide or as DNA constructs design for the expression of suitable protein/polypeptide/peptide. Effective portions of the full-length KDR or Tek proteins will be advantageous in that the full-length sequences have homology to a range of other tyrosine kinases. The truncated forms will have the epitopes identified as being associated with helper and cytotoxic T cell responses. The use of minigenes provides a rapid easy means of generating vaccines. Bicistronic constructs can be readily designed in which for example both CTL and helper epitopes are carried on a single plasmid.

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Where T cell epitopes expressed by endothelial receptors stimulate either helper and/or cytotoxic T lymphocytes which recognise and kill endothelial cells overexpressing these antigens, then the approach is tested for efficacy and potential toxicity in a mouse model. T cell epitopes which bind to both human HLA-A24 and Balb/c H-2K^d may be used as both these sets of alleles have similar requirements for T cell epitope binding (Brusic, et al., 1994 supra). These epitopes may also represent regions of the receptors which show homology between the mouse and human proteins. If this is not possible then peptides binding to mouse MHC representing protein sequences from the respective mice receptors are identified in accordance with the disclosures herein.

Mice are immunised with the constructs outlined above. CTL and blastogenesis responses are measured. Mice carrying tumours are immunised to test for therapeutic effects and the potential toxic effect on wound healing assessed. Candidate vaccines are tested in phase I clinical trials.